

UNCLASSIFIED

AD NUMBER
AD459386
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Mar 1965. Other requests shall be referred to Commanding Officer, US Army Biological Laboratories, Attn: TRB/TID, Fort Detrick, Frederick, MD 21701.
AUTHORITY
BDRL, D/A ltr, 28 Sep 1971

THIS PAGE IS UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

CLASSIFIED BY: DDC

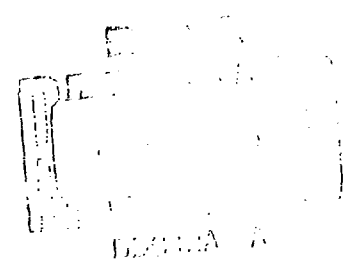
AS AD NO. 459386

TECHNICAL MANUSCRIPT 206

9-AMINOACRIDINE BINDING
TO DEOXYRIBONUCLEIC ACID:
A FLUOROMETRIC ANALYSIS

4 5 9 3 8 6

MARCH 1965



UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 206

9-AMINOACRIDINE BINDING TO DEOXYRIBONUCLEIC ACID:
A FLUOROMETRIC ANALYSIS

Robert E. Boyle

Physical Defense Division
DIRECTORATE OF MEDICAL RESEARCH

Project 1C622401A071

March 1965

This publication or any portion thereof may not be reproduced without specific authorization from the Commanding Officer, U. S. Army Biological Laboratories, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland. 21701. However, DDC is authorized to reproduce the publication for U. S. Government purposes.

The information in this publication has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this publication directly from DDC.

Foreign announcement and dissemination of this publication by DDC is limited.

ABSTRACT

By using fluorescence quenching and a modification of a spectrophotometric calculation, the number of binding sites for 9-aminoacridine on deoxyribonucleic acid was determined to be 650. That number remained constant although the intrinsic association constant varied from 7.0×10^8 to 0.17×10^8 as a function of ionic strength, pH, and dye concentration.

9-AMINOACRIDINE BINDING TO DEOXYRIBONUCLEIC ACID:
A FLUOROMETRIC ANALYSIS

The binding of dyes to polymers has been described by the Langmuir absorption isotherm:

$$r = \frac{nc}{K + c} \quad (1)$$

where r is the number of molecules of dye bound to a molecule of polymer, n is the number of binding sites, c is the equilibrium concentration of the free dye, and K is the intrinsic dissociation constant of the complex. There are many experimental techniques for arriving at the value of K for a given system.¹ One such method is the spectrophotometric method described by Clark and Shack^{2,3} modified by Irvin, Irvin, and Parker⁴ and further modified by Peacocke and Skerrett.⁵

This paper presents a further modification that utilizes fluorescence quenching measurements.

Let P equal the molar concentration of the polymer, DNA in this case, I the initial molar concentration of the ligand 9-aminoacridine, c the equilibrium concentration of the ligand, F_i the fluorescence of I , F_b the fluorescence of the bound I at maximum quenching (in the presence of excess polymer), and F the fluorescence of the mixture at any other given value of P . Then:

$$F = \frac{cF_i}{I} + F_b \frac{rP}{I} \quad (2)$$

Defining α as the fraction of total dye bound, then:

$$\alpha = rP/I = (F_i - F)/(F_i - F_b) \quad (3)$$

Knowing α , then:

$$r = \alpha I/P \quad (4)$$

and

$$c = I(1-\alpha) \quad (5)$$

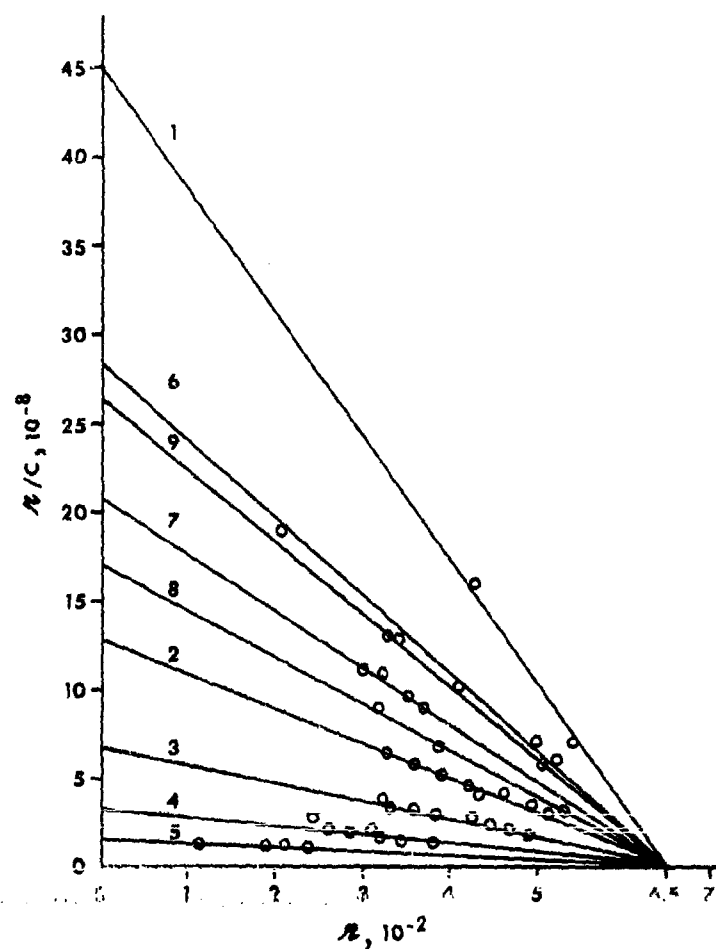
From these values and the values of P and I , the constants K and n can be evaluated from the graphs of r/c versus r , where the intercept on the ordinate is Kn , on the abscissa is n , and the slope of the line is $-K$.

The 9-aminoacridine used in this study was obtained through the courtesy of Professor Adrian Albert of the Australian National University and was used without further treatment. It had an absorption maximum at 400 m μ and an emission maximum at 500 m μ . The absorption maximum was determined on a Beckman DK2 spectrophotometer and all fluorescence measurements were made on a Farrand spectrophotofluorometer at room temperature with 1-cm quartz cells. Highly polymerized grade A salmon sperm DNA (California Corp. for Biochemical Research) was used throughout these studies without further treatment. It had a molecular weight of 1.2×10^6 as determined by light-scattering studies. The solvent system was sodium acetate - sodium barbital buffer with pH and ionic strength adjusted as desired with HCl or NaCl.

Results of plotting r/c versus r as a function of ionic strength, pH, and dye concentration are shown in Figure 1, with the values of K and n .

The homogeneity of the binding is indicated by the constancy of the number of binding sites. The variations in the intrinsic association constants are in complete agreement with previously reported studies,⁵⁻⁸ although the absolute values do differ. This might be due to the degree of polymerization of the deoxyribonucleic acid. The low value for K at pH 9.0 is probably associated with depolymerization or denaturation or both.

Application of the method used here is suggested for dye-binding studies in dilute solution where the binding is homogeneous, and for elimination of the errors associated with dye loss on surfaces of material such as viscose tubing used in binding studies.



Curve	Dye Concentration, 10^{-6} M	pH	Ionic Strength, M	$K, 10^6$	$n, 10^3$
1	3	7.0	0.005	7.00	6.5
2	3	7.0	0.010	2.00	6.5
3	3	7.0	0.025	1.04	6.5
4	3	7.0	0.050	0.53	6.5
5	3	7.0	0.100	0.17	6.5
6	1	7.0	0.005	4.38	6.5
7	0.5	7.0	0.005	3.20	6.5
8	3	5.0	0.005	2.62	6.5
9	3	9.0	0.005	3.82	6.5

Figure 1. Binding of 9-Aminoacridine to DNA, r/c versus r .

LITERATURE CITED

1. Rosenberg, R.M., and I.M. Klotz. 1960. Dye-binding methods, p. 133-168. In P. Alexander and R.J. Block (ed.) Laboratory manual of analytical methods of protein chemistry: Vol. 2, Composition, structure and reactivity of proteins. Pergamon Press, New York.
2. Clark, W.M., J.F. Taylor, T.H. Davies, and C.S. Vestling. 1940. Metalloporphyrine: I. Coordination with nitrogenous bases, theoretical relations. J. Biol. Chem. 135:543-568.
3. Shack, J., and W.M. Clark. 1943. Metalloporphyrine: VI. Cycles of changes in systems containing heme. J. Biol. Chem. 171:143-187.
4. Irvin, L., E.M. Irvin, and F.S. Parker. 1949. The interaction of antimalarials with nucleic acids: I. Acridines. II. Quinolines. Science 110:426-428.
5. Peacocke, A.R., and J.N.H. Skerrett. 1956. The interaction of amino acridines with nucleic acids. Trans. Faraday Soc. 52:261-279.
6. Oster, G. 1955. Dye binding to high polymers. J. Polymer Sci. 16:235-244.
7. Lawley, P.D. 1956. Interaction studies with DNA: IV. The binding of 5-aminoacridine studied fluorometrically, and its comparison with the binding of 9-aminoacridine. Biochem. Biophys. Acta 22:451-458.
8. Irvin, L., and E.M. Irvin. 1954. The interaction of a 9-amino-acridine derivative with nucleic acids and nucleoproteins. J. Biol. Chem. 206:39-49.

DISTRIBUTION LIST

<u>ADDRESSEE</u>	<u>NUMBER OF COPIES</u>
Assistant Scientific Director Building 812	1
Directorate of Biological Research Building 560	1
Directorate of Industrial Health & Safety Building 550	1
Acting Director of Medical Research Building 538	1
Chief, Program Coordination Office Building 825	1
Chief, Aerobiology Division Building 459	1
Chief, Biomathematics Division Building 1422	1
Chief, Medical Bacteriology Division Building 560	1
Chief, Medical Investigation Division Building 604	1
Chief, Physical Sciences Division Building 568	2
Chief, Process Development Division Building 469	1
Chief, Technical Evaluation Division Building 568	1
Test Chamber Branch Technical Evaluation Division Building 1412	1
Documents, Technical Library Building 426	2
Technical Releases Branch Technical Information Division Building 426	10

<u>ADDRESSEE</u>	<u>NUMBER OF COPIES</u>
Editorial Branch Technical Information Division Building 816	1
U.S. Army Medical Unit Building 120	1
Liaison Representative Animal Disease Investigations Building 1301	1
U.S. Public Health Service Liaison Office Building 1301	6
Commanding Officer U.S. Naval Unit Building 125	3
Commanding General U.S. Army Edgewood Arsenal ATTN: SMUEA-CS Edgewood Arsenal, Maryland, 21010	1
Commanding Officer U.S. Army Chemical Research & Development Laboratories ATTN: Librarian Edgewood Arsenal, Maryland, 21010	2
Commanding General U.S. Army Munitions Command ATTN: AMSMU-CS Dover, New Jersey, 07801	1
Commanding General U.S. Army Munitions Command ATTN: AMSMU-RE-RR, Mr. G. Chesnov Dover, New Jersey, 07801	1
Commandant U.S. Army CBR Weapons Orientation Course Dugway Proving Ground Dugway, Utah, 84022	1
Commanding General Deseret Test Center ATTN: Technical Library Fort Douglas, Utah, 84113	2

<u>ADDRESSEE</u>	<u>NUMBER OF COPIES</u>
Commanding General U.S. Army Materiel Command Research Division, AMCRD-RC R&D Directorate Washington, D.C., 20315	1
Defense Documentation Center Cameron Station Alexandria, Virginia, 22314	20
AFRSTA, Hq. USAF ATTN: Mr. C.R. Nixon, Jr. Washington, D.C., 20330	1
Detachment 4, RTD (ATCB) Eglin AFB, Florida, 32542	1
APGC (PGBAP-1) Eglin AFB, Florida, 32542	1
6570 AMRL MRMP13 (Dr. S.A. London) Wright-Patterson Air Force Base, Ohio, 45433	1
Dr. S.H. Madin Scientific Director Naval Biological Laboratory Naval Supply Center Oakland 14, California, 94614	1
Commander (Code 4036) U.S. Naval Ordnance Test Station China Lake, California, 93557	1
Commanding Officer and Director U.S. Naval Applied Science Laboratory Naval Base, Code 9440 Brooklyn, New York, 11251	1
U.S. Army Medical R&D Command Office of the Surgeon General ATTN: MEDDH-C Room 2526, Main Navy Building Washington, D.C., 36205	1

ADDRESSEENUMBER OF COPIES

Commandant USACmICen & Sch, ATTN: Bio Br Ft. McClellan, Alabama, 36205	1
U.S. Army Standardization Group - Canada Office, Senior Standardization Rep. c/o Director of Equipment Policy Canadian Army Headquarters Ottawa 4, Canada	1
Munitions/TW Defence Research Staff British Embassy 3100 Massachusetts Avenue, N.W. Washington 8, D.C.	3
Canadian Liaison Office (CBR) Building 5101 Edgewood Arsenal, Maryland, 21010	3
Australian Embassy ATTN: Lt. Col. P.D. Yonge Australian Army Staff (W) 2001 Connecticut Avenue, N.W. Washington 7, D.C.	2
Dr. Robert E. Boyle Physical Defense Division Building 521	10